Figure 1

gp120

MIVTMRAMGK RNRKLGILYI VMALIIPCLS SSQLYATVYA GVPVWEDAAP 50

VLFCASDANL TSTEKHNVWA SQACVPTDPT PHEYLLTNVT DNFNIWENYM 100

VEQMQEDIIS LWDQSLKPCI QMTFMCIQMN CTDIKNNNTS GTENRTSSSE 150

NPMKTCEFNI TTVLKDKKEK KQALFYVSDL TKLADNNTTN TMYTLINCNS 200

TTIKQACPKV SFEPIPIYYC APAGYAIFKC NSAEFNGTGK CSNISVVTCT 250

HGIKPTVSTQ LILNGTLSKE KIRIMGKNIS DSGKNIIVTL SSDIEITCVR 300

PGNNQTVQEM KIGPMAWYSM ALGTGSNRSR VAYCQYNTTE WEKALKNTAE 350

RYLELINNTE GNTTMIFNRS QDGSDVEVTH LHFNCHGEFF YCNTSEMFNY 400

TFLCNGTNCN NTQSINSANG MIPCKLKQVV RSWMRGGSGL YAPPIPGNLT 450

CISHITGMIL QMDAPWNKTE NTFRPIGGDM KDIWRNELFK YKVVRVKPFS 500

gp41

VAPTPIARPV IGTGTHREKR AVGLGMLFLG VLSAAGSTMG AAATALTVQT 550

HSVIKGIVQQ QDNLLRAIQA QQELLRLSVW GIRQLRARLL ALETLIQNQQ 600

LLNLWGCKGR LICYTSVKWN ETWRNTTNIN QIWGNLTWQE WDQQIDNVSS 650

TIYEEIQKAQ VQQEQNEKKL LELDEWASLW NWLDITKWLW YIKIAIIIVG 700

ALIGVRIVMI VLNLVRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGDE 750

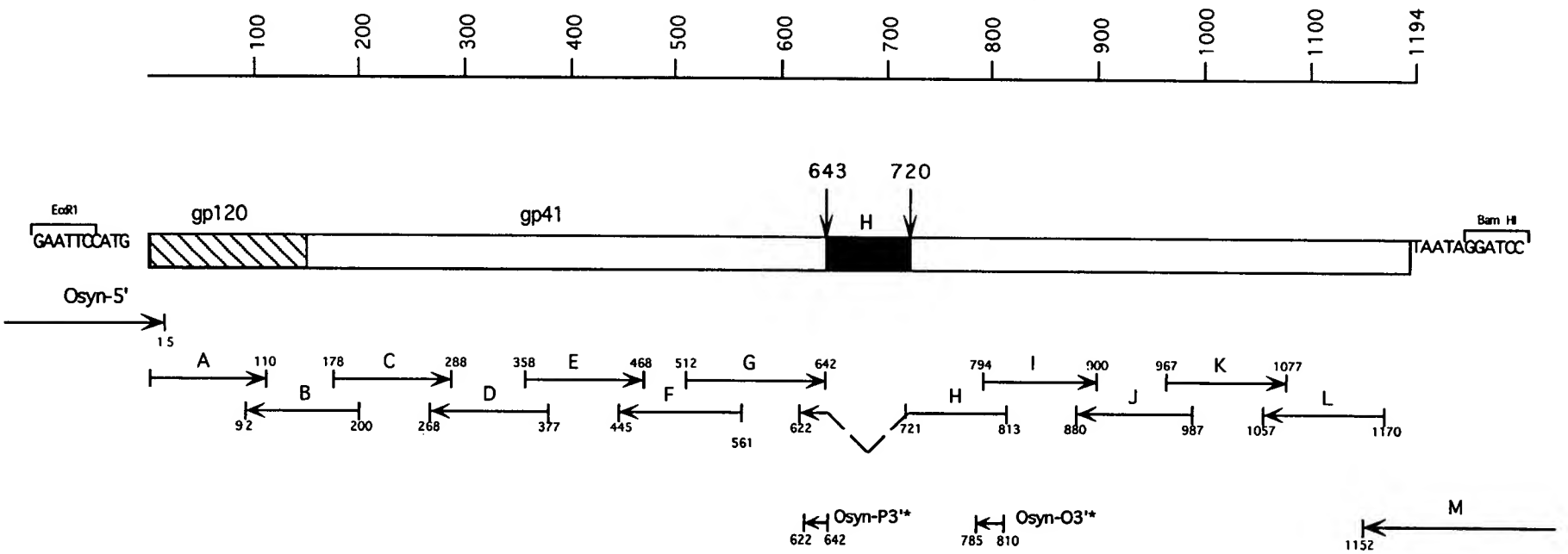
GRPRLIPSPQ GFLPLLYTDL RTIILWSYHL LSNLISGTQT VISHLRLGLW 800

ILGQKIIDAC RICAAVIHYW LQELQKSATS LIDTFAVAVA NWTDDIILGI 850

QRLGRGILNI PRRVRQGFER SLL 873

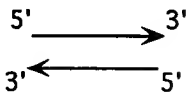
Figure 2

Diagram of Oligonuclotides Used to Generate Synthetic Gene Constructs



* Translational terminators and
Bam HI cloning sites on the 5' end

pGO-8 insert = Osyn-5' to Osyn-P3'
pGO-9 insert = Osyn-5' to Osyn-O3'
pGO-11 insert = Osyn-5' to Osyn-M
H = Hydrophobic region (deleted as shown)



Construction of pGO-9PL/DH5α and pGO-9CKS/XL1

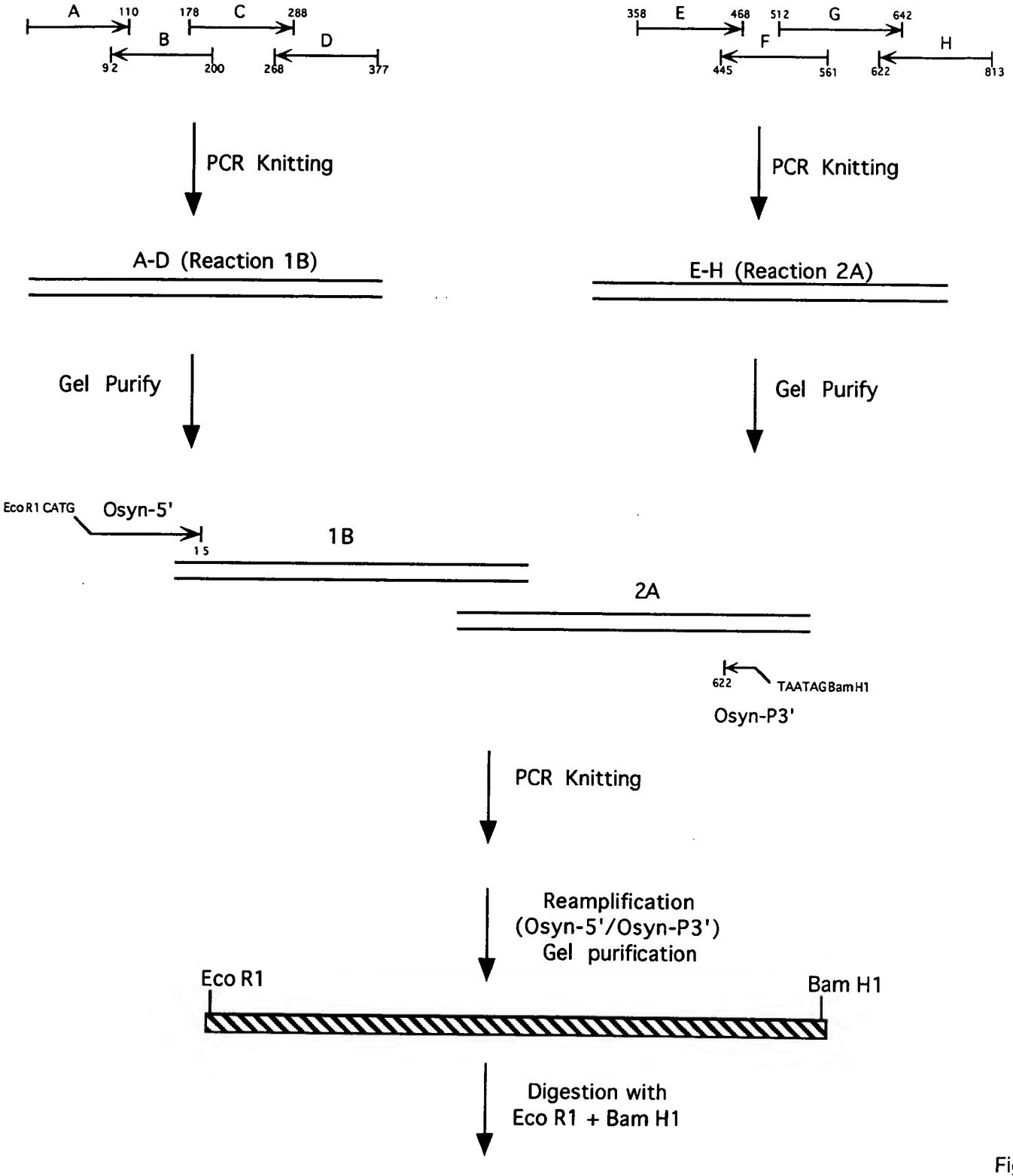


Fig 3A

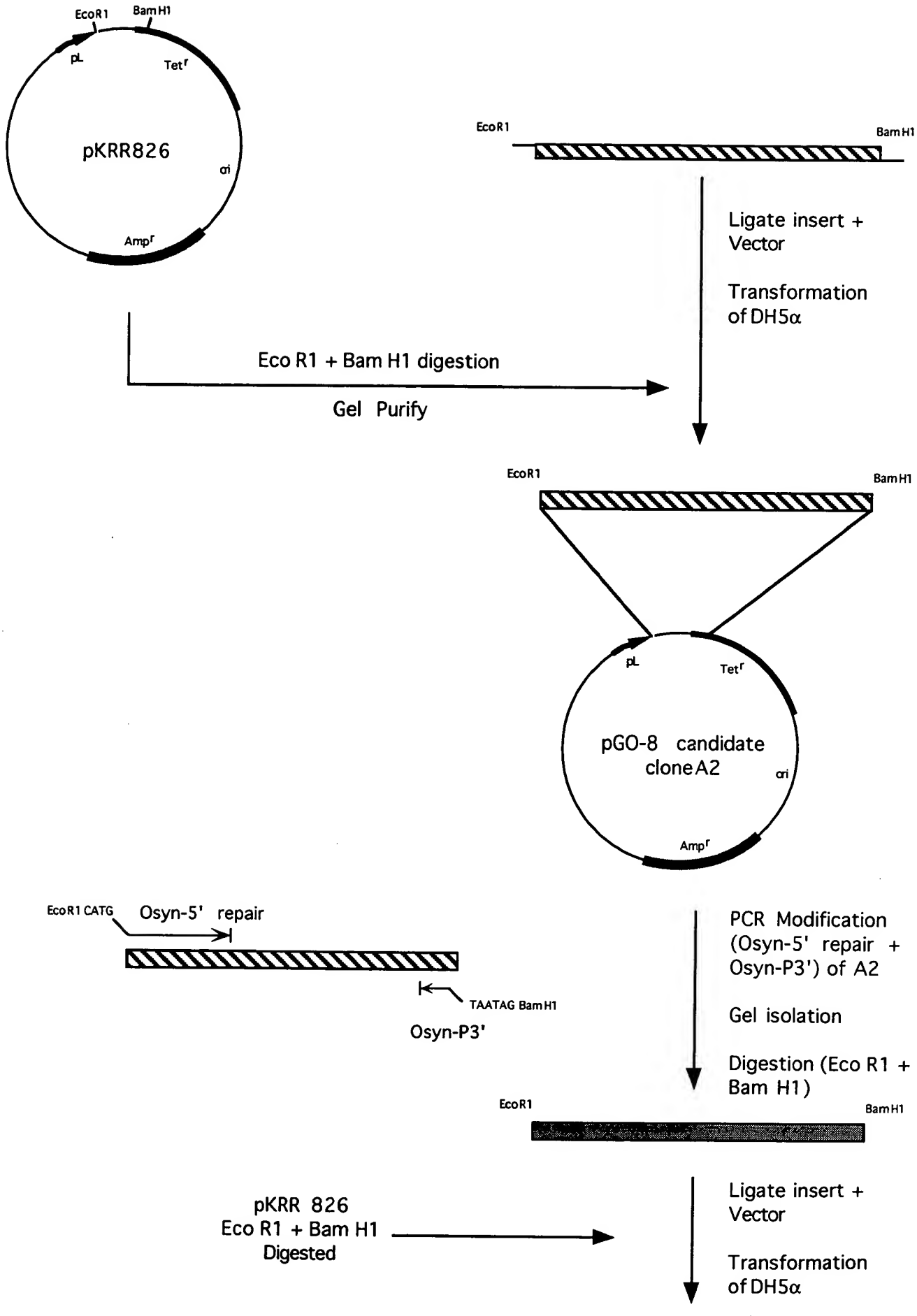

EcoR1
Bam H1
pL
Tet^r
pKRR826
ori
Amp^r
EcoR1
Bam H1
Ligate insert + Vector
Transformation of DH5α
Eco R1 + Bam H1 digestion
Gel Purify
EcoR1
Bam H1
pL
Tet^r
pGO-8 candidate clone A2
ori
Amp^r
EcoR1 CATG
Osyn-5' repair
TAATAG Bam H1
Osyn-P3'
PCR Modification (Osyn-5' repair + Osyn-P3') of A2
Gel isolation
Digestion (Eco R1 + Bam H1)
EcoR1
Bam H1
pKRR 826
Eco R1 + Bam H1
Digested
Ligate insert + Vector
Transformation of DH5α

Fig 3B

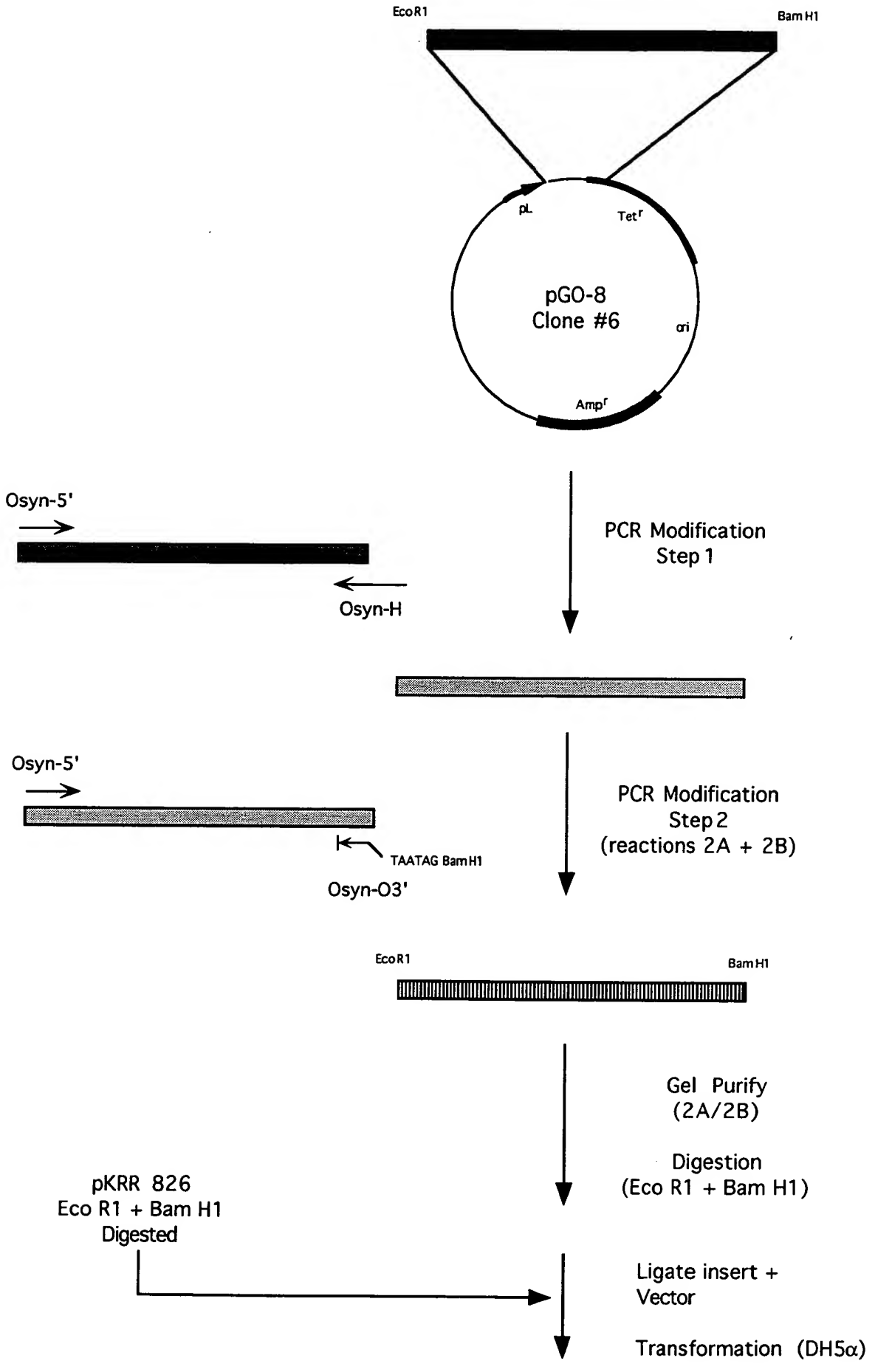

EcoR1
BamH1
pL
Tet^r
pGO-8
Clone #6
ori
Amp^r
Osyn-5'
Osyn-H
PCR Modification
Step 1
Osyn-5'
TAATAG BamH1
Osyn-O3'
PCR Modification
Step 2
(reactions 2A + 2B)
EcoR1
BamH1
Gel Purify
(2A/2B)
Digestion
(Eco R1 + Bam H1)
pKRR 826
Eco R1 + Bam H1
Digested
Ligate insert +
Vector
Transformation (DH5α)

Fig 3C

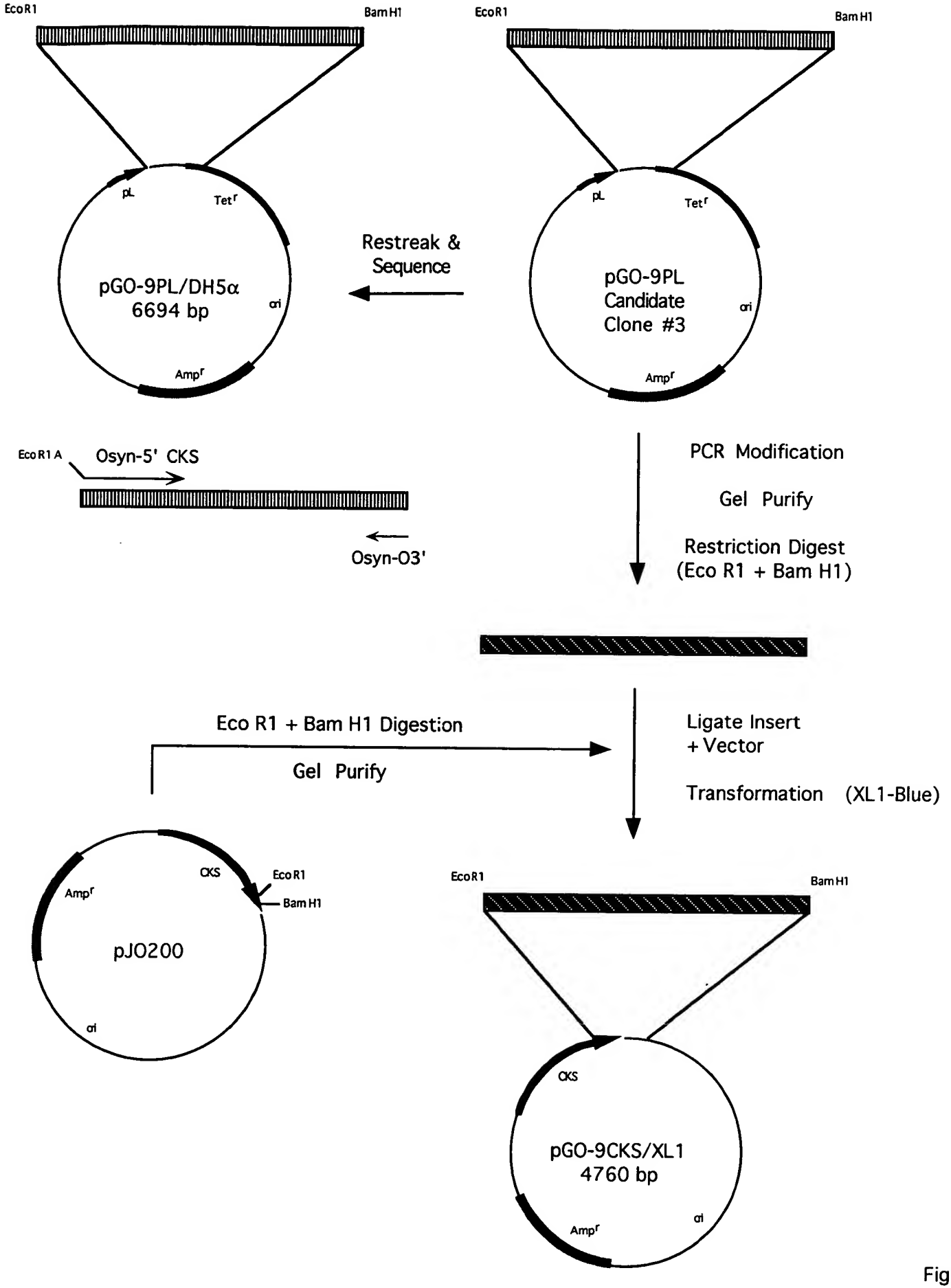

EcoR1
BamH1
pL
Tet^r
pGO-9PL/DH5α
6694 bp
ori
Amp^r
Restreak &
Sequence
EcoR1
BamH1
pL
Tet^r
pGO-9PL
Candidate
Clone #3
ori
Amp^r
EcoR1 A
Osyn-5' CKS
Osyn-03'
PCR Modification
Gel Purify
Restriction Digest
(Eco R1 + Bam H1)
Eco R1 + Bam H1 Digestion
Gel Purify
Ligate Insert
+ Vector
Transformation (XL1-Blue)
Amp^r
CKS
EcoR1
Bam H1
pJO200
ori
EcoR1
BamH1
CKS
pGO-9CKS/XL1
4760 bp
Amp^r
ori

Fig 3D

Construction of pGO-11PL and pGO-11CKS Synthetic Genes

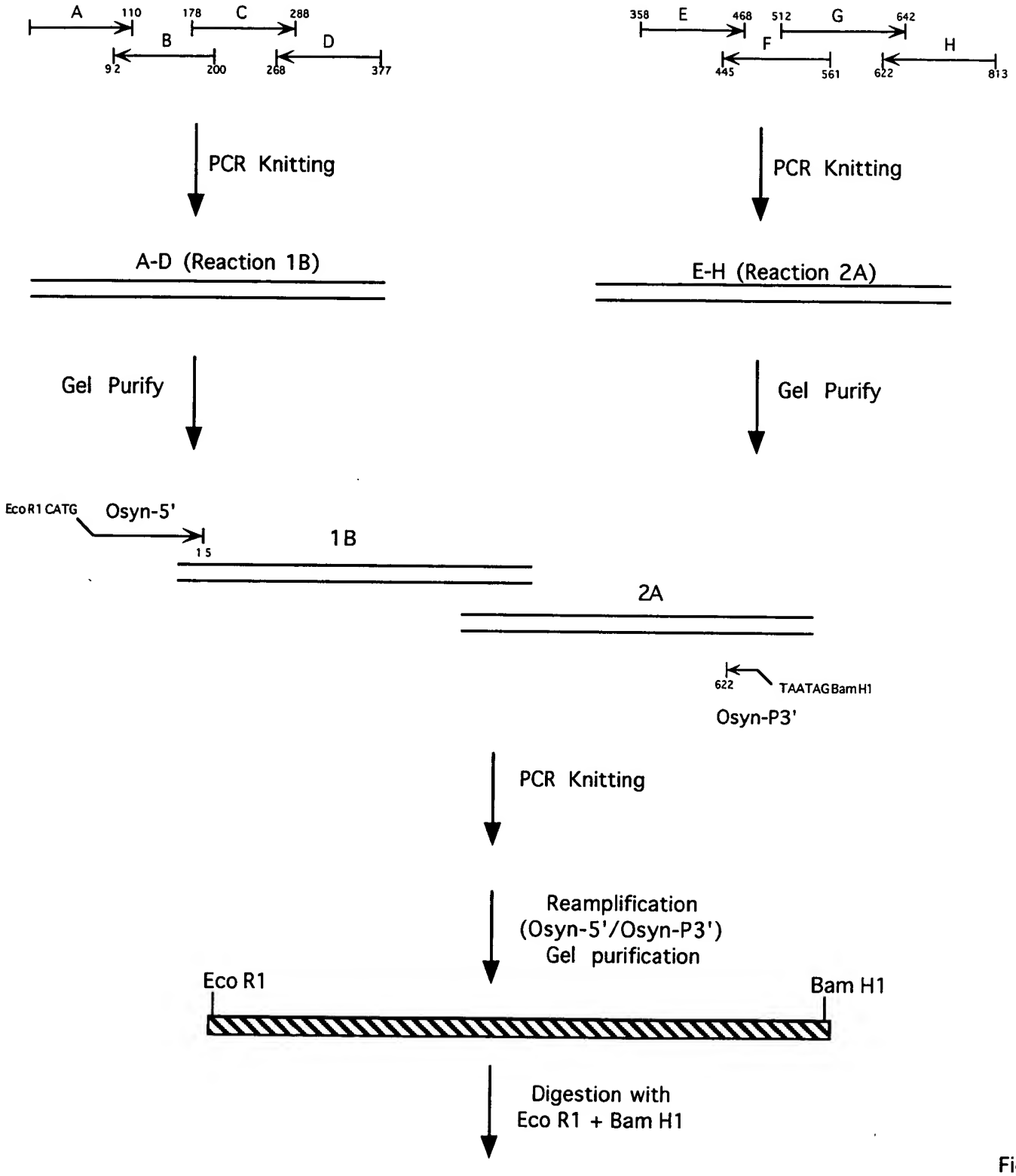


Fig 4A

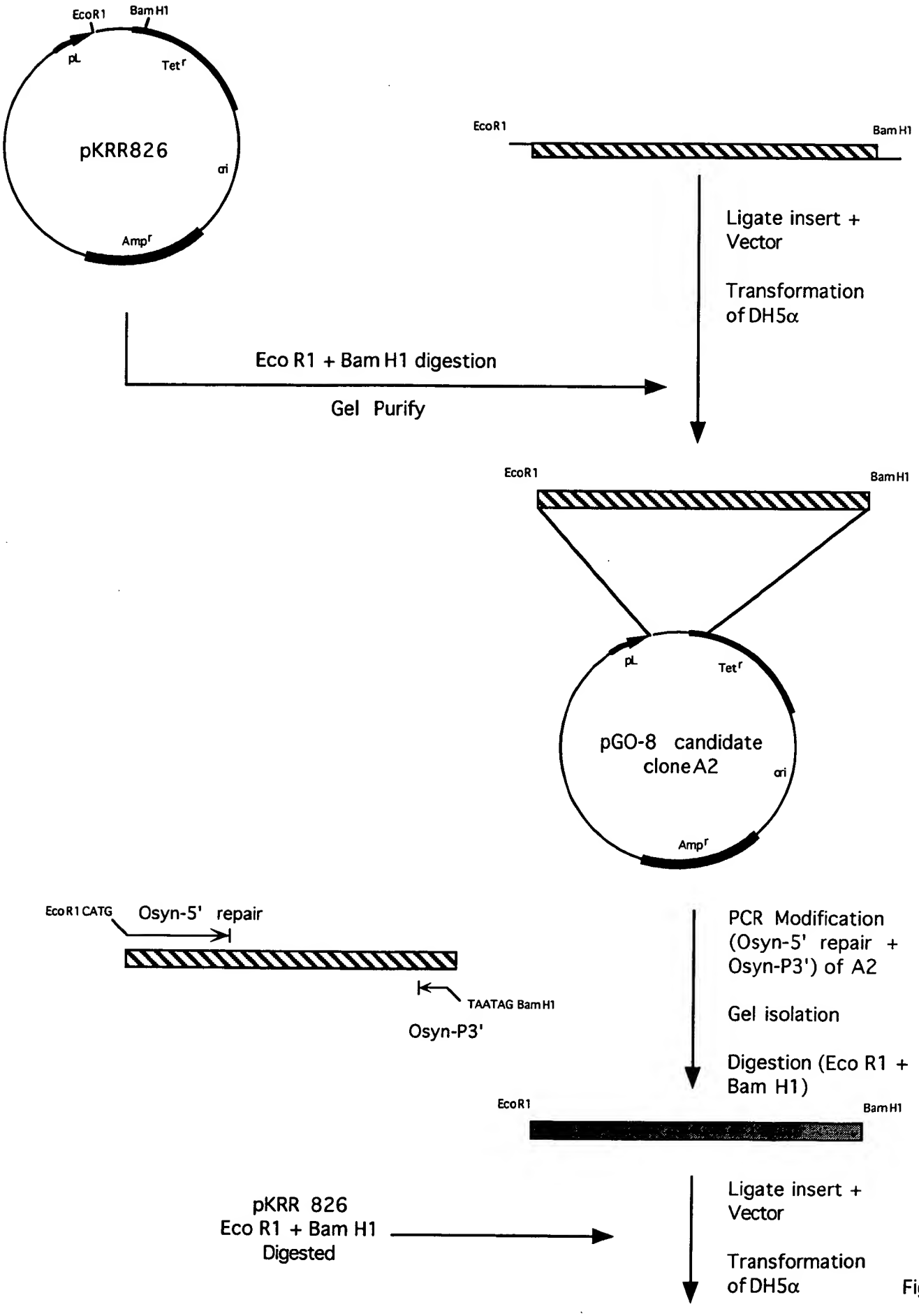

EcoR1
Bam H1
pL
Tet^r
pKRR826
ori
Amp^r
EcoR1
Bam H1
Ligate insert + Vector
Transformation of DH5α
Eco R1 + Bam H1 digestion
Gel Purify
EcoR1
BamH1
pL
Tet^r
pGO-8 candidate clone A2
ori
Amp^r
EcoR1 CATG
Osyn-5' repair
TAATAG Bam H1
Osyn-P3'
PCR Modification (Osyn-5' repair + Osyn-P3') of A2
Gel isolation
Digestion (Eco R1 + Bam H1)
EcoR1
Bam H1
pKRR 826
Eco R1 + Bam H1
Digested
Ligate insert + Vector
Transformation of DH5α

Fig 4B

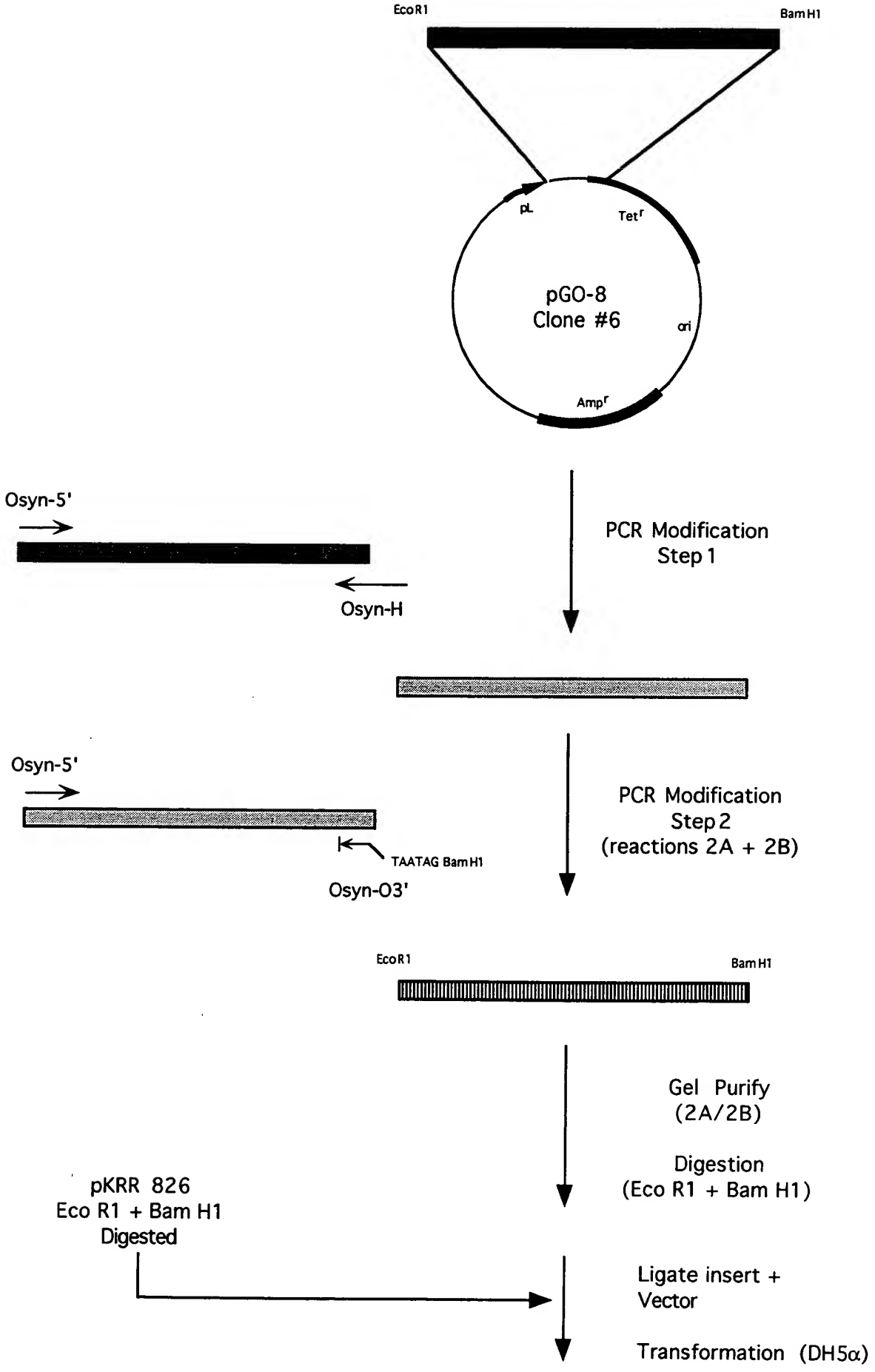
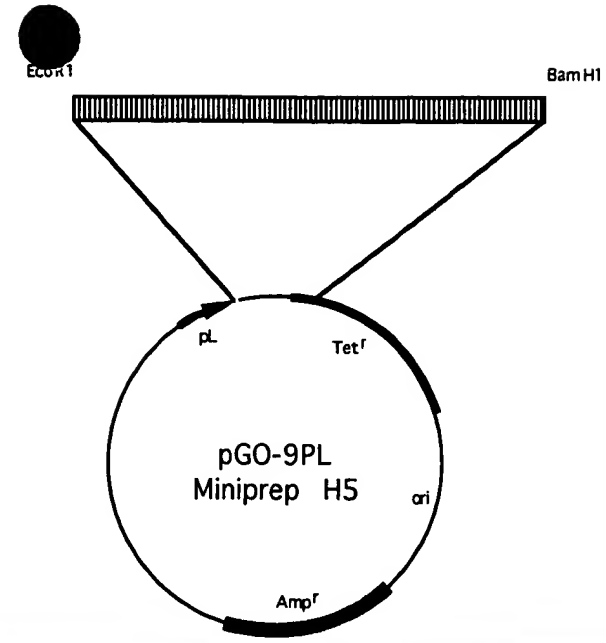

EcoR1
BamH1
pL
Tet^r
pGO-8
Clone #6
ori
Amp^r
Osyn-5'
Osyn-H
PCR Modification
Step 1
Osyn-5'
TAATAG BamH1
Osyn-03'
PCR Modification
Step 2
(reactions 2A + 2B)
EcoR1
BamH1
Gel Purify
(2A/2B)
Digestion
(Eco R1 + Bam H1)
pKRR 826
Eco R1 + Bam H1
Digested
Ligate insert +
Vector
Transformation (DH5α)

Fig 4C


EcoR1
BamH1
pL
Tet^r
pGO-9PL
Miniprep H5
ori
Amp^r

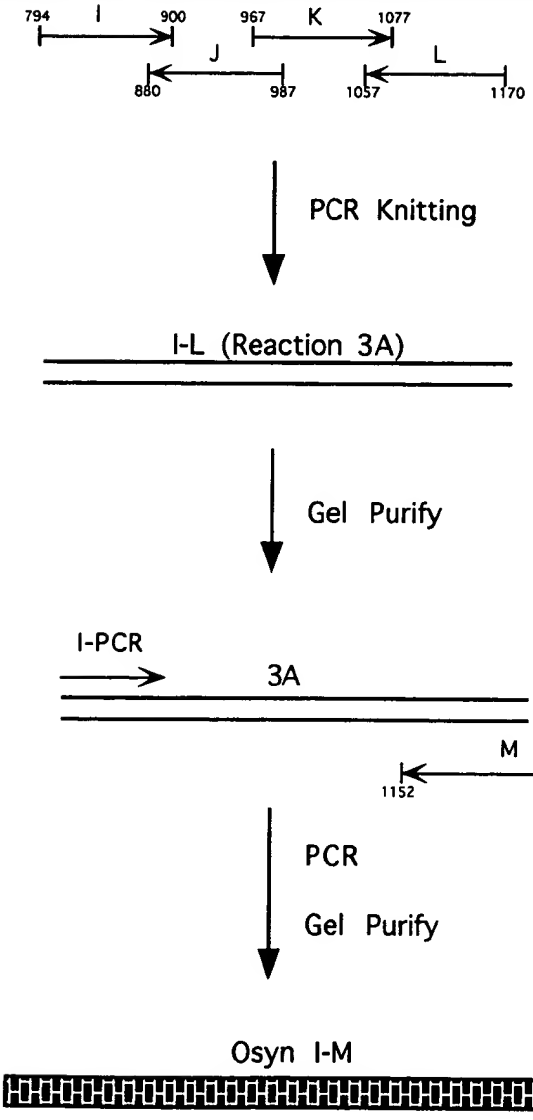

794
I
900
967
K
1077
J
880
987
L
1057
1170
PCR Knitting
I-L (Reaction 3A)
Gel Purify
I-PCR
3A
M
1152
PCR
Gel Purify
Osyn I-M

Fig 4D

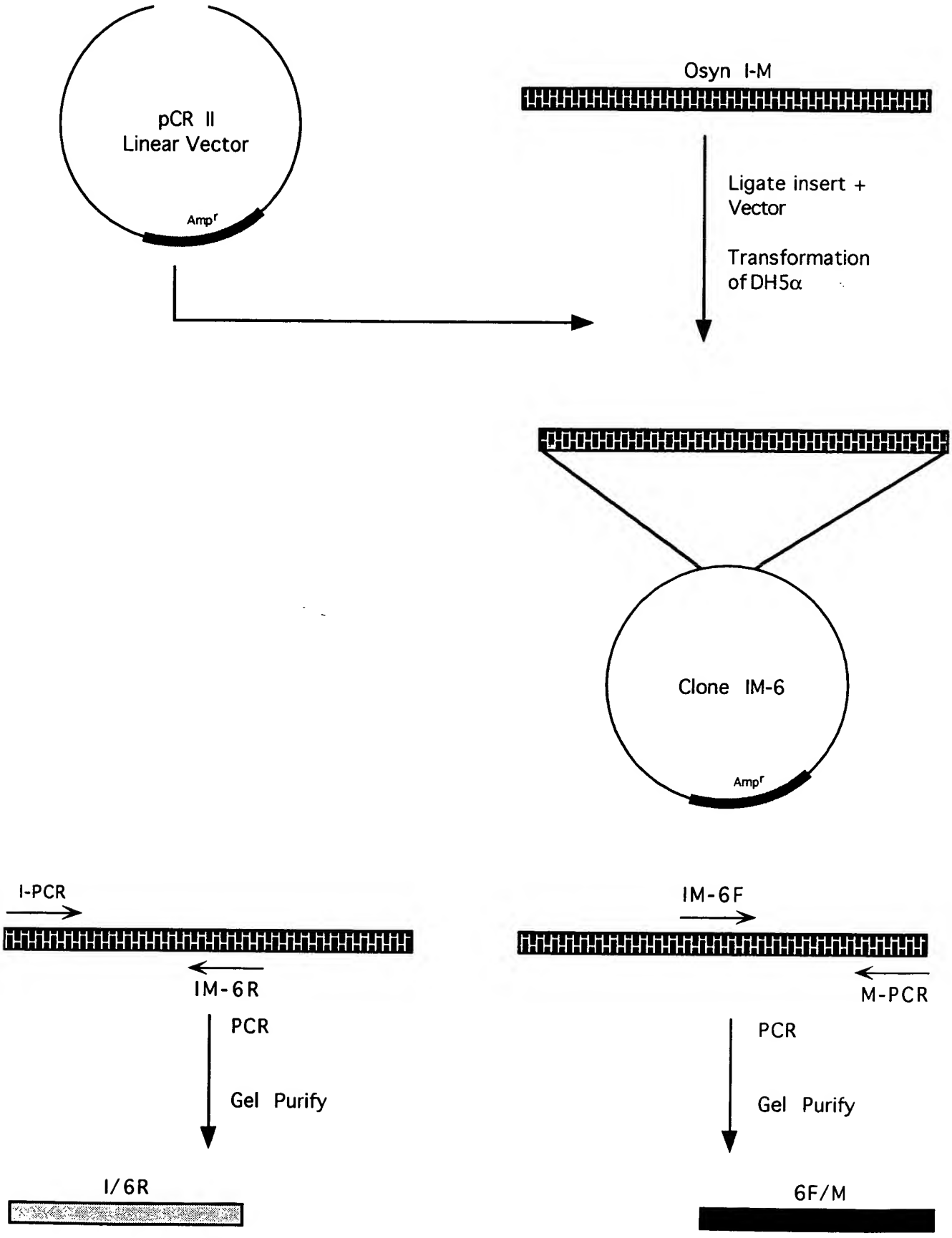


Fig 4E

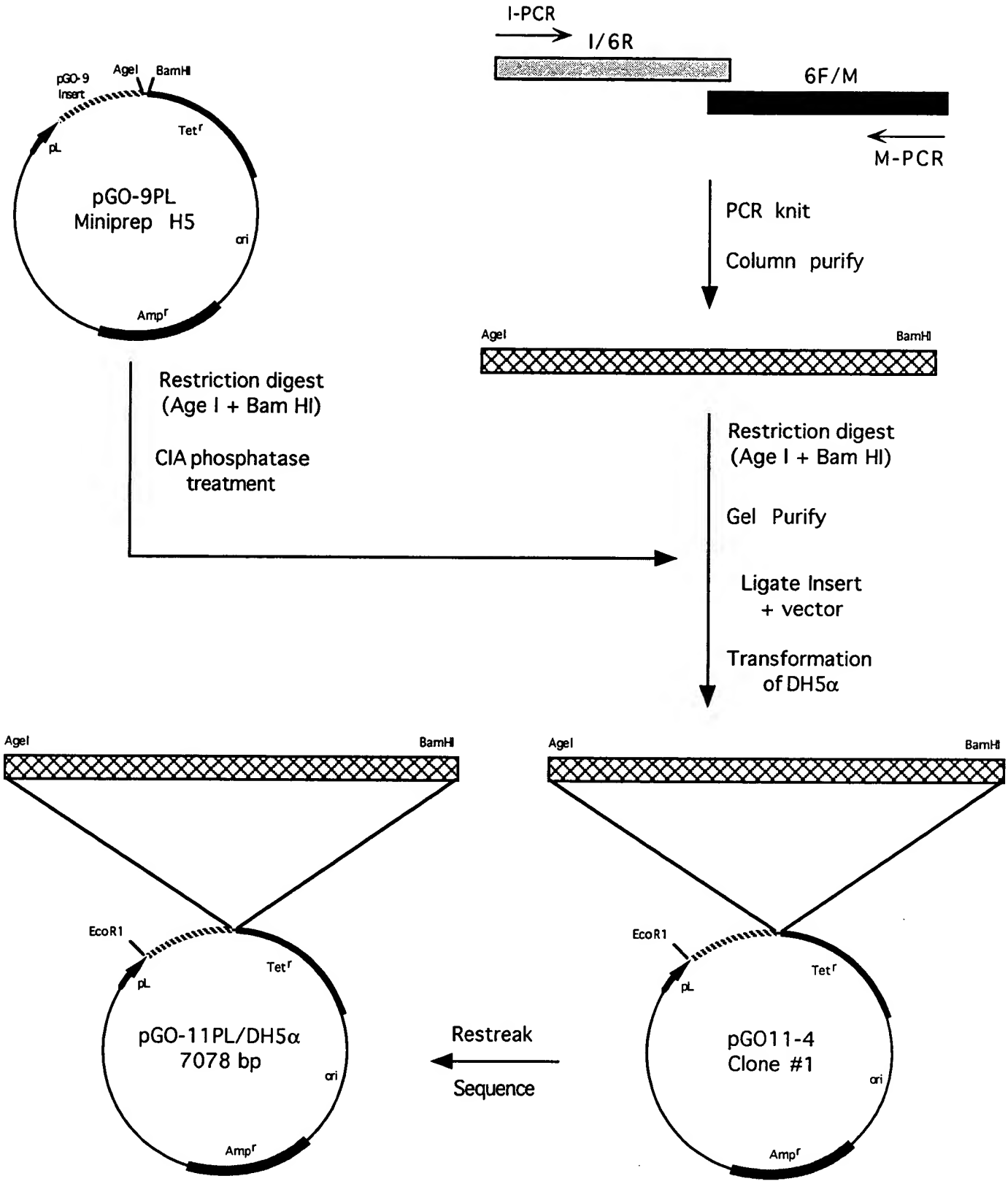

I-PCR
I/6R
6F/M
M-PCR
pGO-9 Insert
AgeI
BamHI
Tet^r
pL
pGO-9PL
Miniprep H5
ori
Amp^r
PCR knit
Column purify
AgeI
BamHI
Restriction digest
(Age I + Bam HI)
CIA phosphatase
treatment
Restriction digest
(Age I + Bam HI)
Gel Purify
Ligate Insert
+ vector
Transformation
of DH5α
AgeI
BamHI
EcoR1
Tet^r
pL
pGO-11PL/DH5α
7078 bp
ori
Amp^r
Restreak
Sequence
AgeI
BamHI
EcoR1
Tet^r
pL
pGO11-4
Clone #1
ori
Amp^r

Fig 4F

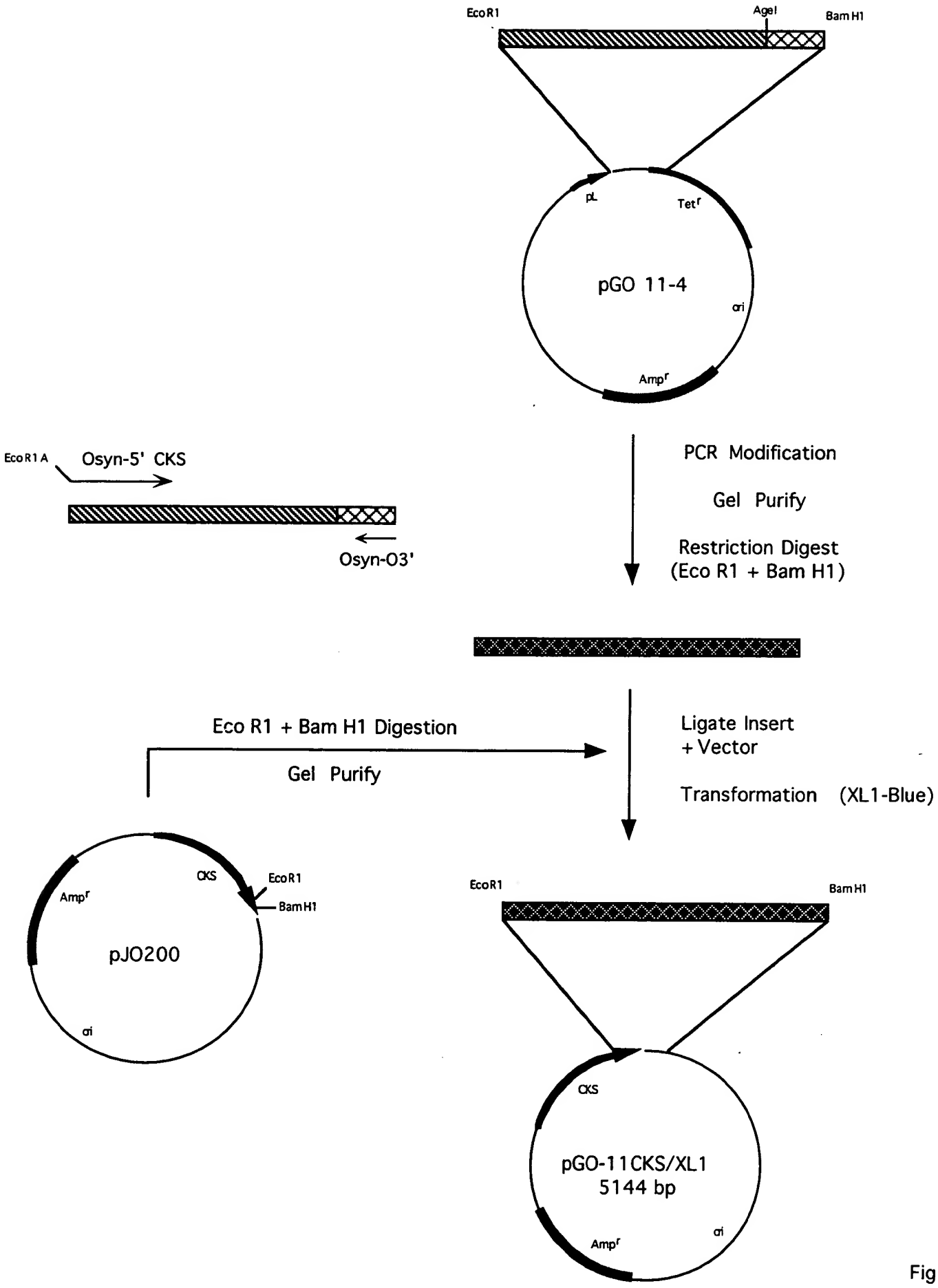

EcoR1
AgeI
BamH1
pL
Tet^r
pGO 11-4
ori
Amp^r
EcoR1 A
Osyn-5' CKS
Osyn-O3'
PCR Modification
Gel Purify
Restriction Digest
(Eco R1 + Bam H1)
Eco R1 + Bam H1 Digestion
Gel Purify
Ligate Insert
+ Vector
Transformation (XL1-Blue)
CKS
EcoR1
BamH1
Amp^r
pJO200
ori
EcoR1
BamH1
CKS
pGO-11CKS/XL1
5144 bp
Amp^r
ori

Fig 4G

Figure 5

gp120 → M ... ; gp41 → A (position 47)

```
MIGGDMKDIW RNELFKYKVV RVKPFSVAPT PIARPVIGTG THREKRAVGL   50

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL  100

LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR  150

NTTNINQIWG NLTWQEWDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD  200

EWASLWNWLD ITKWL                                        215
```

Figure 6

→ CKS

```
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD  50
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100
EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP 200
                                                  → gp120
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDL DPSTNSIGGD 250
                                            → gp41
MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300
GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350
WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400
NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450
WNWLDITKWL                                            460
```

Figure 7

gp120 gp41

MIGGDMKDIW RNELFKYKVV RVKPFSVAPT PIARPVIGTG THREKRAVGL 50

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100

LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR 150

NTTNINQIWG NLTWQEWDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200

EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGE 245

Figure 8

```
┌→ CKS
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD   50

HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD  100

EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG  150

YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP  200
                                                 ┌→ gp120
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDL DPSTNSIGGD  250
                                             ┌→ gp41
MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL  300

GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV  350

WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI  400

NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL  450

WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE             490
```

Figure 9

gp120 gp41

MIGGDMKDIW RNELFKYKVV RVKPFSVAPT PIARPVIGTG THREKRAVGL 50

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100

LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR 150

NTTNINQIWG NLTWQEWDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200

EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGDE 250

GRPRLIPSPQ GFLPLLYTDL RTIILWSYHL LSNLISGTQT VISHLRLGLW 300

ILGQKIIDAC RICAAVIHYW LQELQKSATS LIDTFAVAVA NWTDDIILGI 350

QRLGRGILNI PRRVRQGFER SLL 373

Figure 10

CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD 50

HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100

EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150

YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP 200

gp120

SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDL DPSTNSIGGD 250

gp41

MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300

GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350

WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400

NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450

WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE GGGDEGRPRL 500

IPSPQGFLPL LYTDLRTIIL WSYHLLSNLI SGTQTVISHL RLGLWILGQK 550

IIDACRICAA VIHYWLQELQ KSATSLIDTF AVAVANWTDD IILGIQRLGR 600

GILNIPRRVR QGFERSLL 618

Figure 11

CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD 50

HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100

EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150

YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP 200

gp120

SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDL DPSTNSMEGE 250

LTCNSTVTSI IANIDSDGNQ TNITFSAEVA ELYRLELGDY KLIEVTPIGF 300

gp36

APTKEKRYSS APVRNKRGVF VLGFLGFLAT AGSAMGAASL TLSAQSRTLL 350

AGIVQQQQQL LDVVKRQQEM LRLTVWGTKN LQARVTAIEK YLKDQAQLNS 400

WGCAFRQVCH TTVPWVNDSL TPDWNNMTWQ EWEKRVHYLE ANISQSLEQA 450

QIQQEKNMYE LQKLNS 466

Figure 12

```
→CKS
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD   50
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD  100
EPMIPATIIR QVADNLAQRQ VGMATLAVPI HNAEEAFNPN AVKVVLDAEG  150
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP  200
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDP STALMKIPGD  250
→gp120                                        →gp41
PGGGDMRDNW RSELYKYKVV KIEPLGVAPT KAKRRVVQRE KRAVGIGALF  300
LGFLGAAGST MGAASMTLTV QARQLLSGIV QQQNNLLRAI EAQQHLLQLT  350
VWGIKQLQAR ILAVERYLKD QQLLGIWGCS GKLICTTAVP WNASWSNKSL  400
EQIWNNMTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWVNR  450
VRQGYSPLSF QTHLPIPRGP DRPEGIEEEG GERDRDRSIR LVNGSLALIW  500
DDLRSLCLFS YHRLRDLLLI VTRIVELLGR RGWEALKYWW NLLQYWSQEL  550
KNSAVSLLNA TAIAVAEGTD RVIEVVQGAY RAIRHIPRRI RQGLERILL   599
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Figure 13

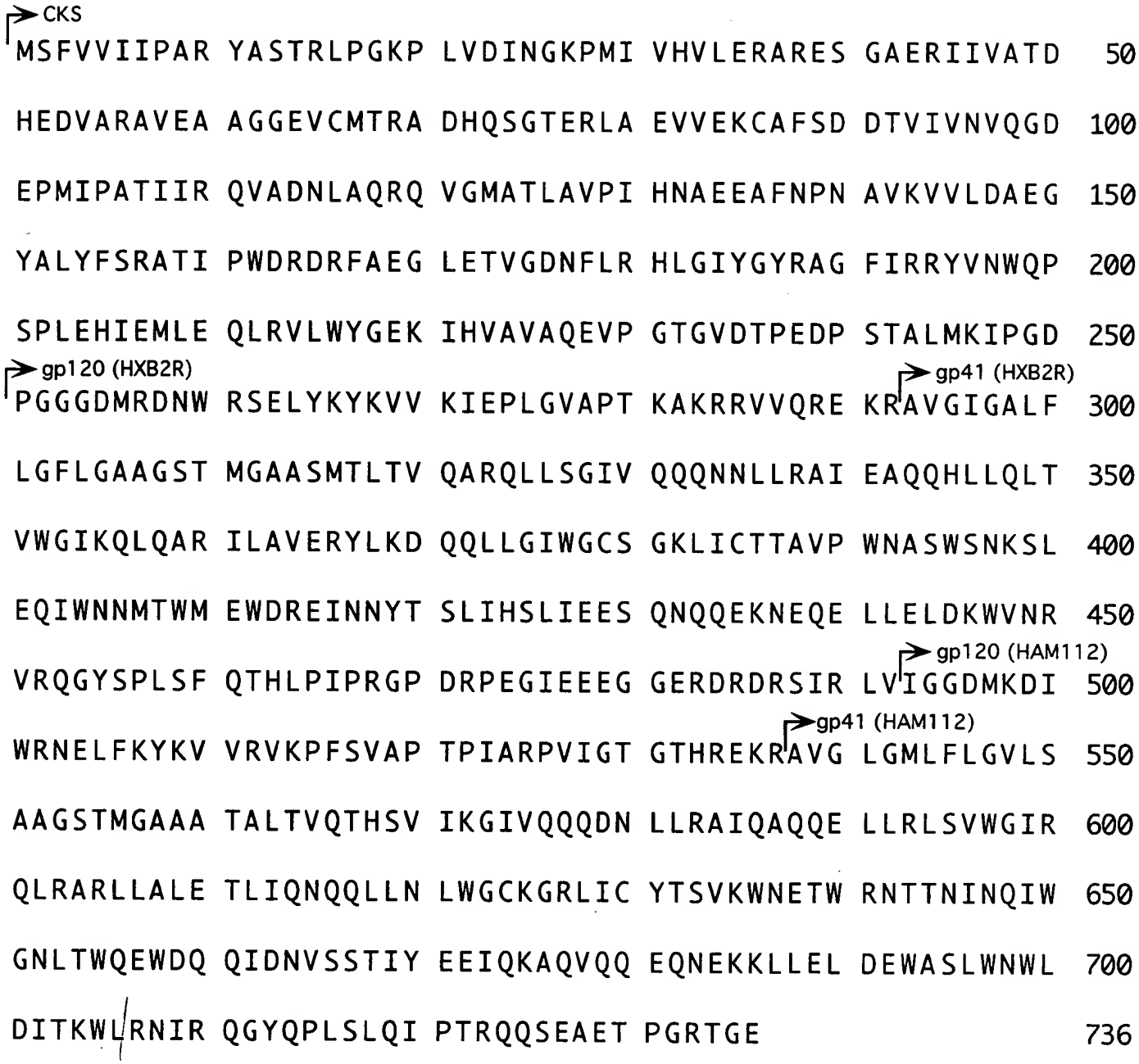
CKS
MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD 50
HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100
EPMIPATIIR QVADNLAQRQ VGMATLAVPI HNAEEAFNPN AVKVVLDAEG 150
YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP 200
SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDP STALMKIPGD 250
gp120 (HXB2R)
PGGGDMRDNW RSELYKYKVV KIEPLGVAPT KAKRRVVQRE KR
gp41 (HXB2R)
AVGIGALF 300
LGFLGAAGST MGAASMTLTV QARQLLSGIV QQQNNLLRAI EAQQHLLQLT 350
VWGIKQLQAR ILAVERYLKD QQLLGIWGCS GKLICTTAVP WNASWSNKSL 400
EQIWNNMTWM EWDREINNYT SLIHSLIEES QNQQEKNEQE LLELDKWVNR 450
gp120 (HAM112)
VRQGYSPLSF QTHLPIPRGP DRPEGIEEEG GERDRDRSIR LVIGGDMKDI 500
gp41 (HAM112)
WRNELFKYKV VRVKPFSVAP TPIARPVIGT GTHREKRAVG LGMLFLGVLS 550
AAGSTMGAAA TALTVQTHSV IKGIVQQQDN LLRAIQAQQE LLRLSVWGIR 600
QLRARLLALE TLIQNQQLLN LWGCKGRLIC YTSVKWNETW RNTTNINQIW 650
GNLTWQEWDQ QIDNVSSTIY EEIQKAQVQQ EQNEKKLLEL DEWASLWNWL 700
DITKWLRNIR QGYQPLSLQI PTRQQSEAET PGRTGE 736

Figure 14

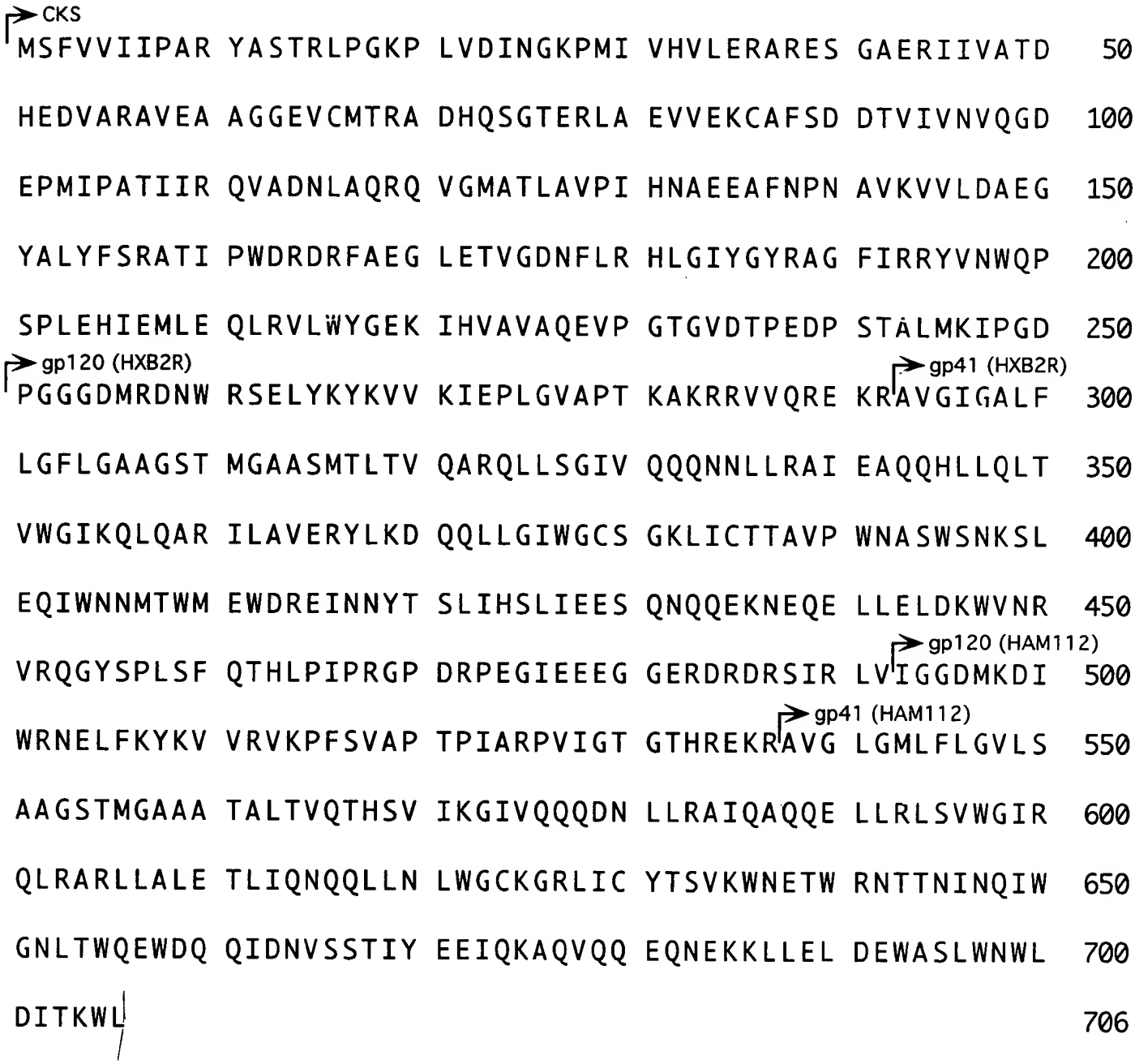

Figure 15

```
 ┌→gp120 (HAM112)                                              ┌→gp41(HAM112)
MIGGDMKDIW RNELFKYKVV RVKPFSVAPT PIARPVIGTG THREKRAVGL  50

GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL 100

LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR 150

NTTNINQIWG NLTWQEWDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD 200
                                                          ┌→gp120 (HXB2R)
EWASLWNWLD ITKWL|RNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGPGGG 250
                                            ┌→gp41 (HXB2R)
DMRDNWRSEL YKYKVVKIEP LGVAPTKAKR RVVQREKRAV GIGALFLGFL 300

GAAGSTMGAA SMTLTVQARQ LLSGIVQQQN NLLRAIEAQQ HLLQLTVWGI 350

KQLQARILAV ERYLKDQQLL GIWGCSGKLI CTTAVPWNAS WSNKSLEQIW 400

NNMTWMEWDR EINNYTSLIH SLIEESQNQQ EKNEQELLEL DKWVNRVRQG 450

YSPLSFQTHL PIPRGPDRPE GIEEEGGERD RDRSIRLV             488
```

Figure 16

→ CKS

MSFVVIIPAR YASTRLPGKP LVDINGKPMI VHVLERARES GAERIIVATD 50

HEDVARAVEA AGGEVCMTRA DHQSGTERLA EVVEKCAFSD DTVIVNVQGD 100

EPMIPATIIR QVADNLAQRQ VGMTTLAVPI HNAEEAFNPN AVKVVLDAEG 150

YALYFSRATI PWDRDRFAEG LETVGDNFLR HLGIYGYRAG FIRRYVNWQP 200

→ gp120 (HAM112)

SPLEHIEMLE QLRVLWYGEK IHVAVAQEVP GTGVDTPEDL DPSTNSIGGD 250

→ gp41 (HAM112)

MKDIWRNELF KYKVVRVKPF SVAPTPIARP VIGTGTHREK RAVGLGMLFL 300

GVLSAAGSTM GAAATALTVQ THSVIKGIVQ QQDNLLRAIQ AQQELLRLSV 350

WGIRQLRARL LALETLIQNQ QLLNLWGCKG RLICYTSVKW NETWRNTTNI 400

NQIWGNLTWQ EWDQQIDNVS STIYEEIQKA QVQQEQNEKK LLELDEWASL 450

→ linker → IDR (HAM112)

WNWLDITKWL RNIRQGYQPL SLQIPTRQQS EAETPGRTGE GGGSRLLALE 500

TLIQNQQLLN LWGCKGRLIC YTSVKW 526

Figure 17

gp120 (HAM112) → (at residue 1); gp41 (HAM112) → (at residue 47)

```
MIGGDMKDIW RNELFKYKVV RVKPFSVAPT PIARPVIGTG THREKRAVGL    50
GMLFLGVLSA AGSTMGAAAT ALTVQTHSVI KGIVQQQDNL LRAIQAQQEL   100
LRLSVWGIRQ LRARLLALET LIQNQQLLNL WGCKGRLICY TSVKWNETWR   150
NTTNINQIWG NLTWQEWDQQ IDNVSSTIYE EIQKAQVQQE QNEKKLLELD   200
EWASLWNWLD ITKWLRNIRQ GYQPLSLQIP TRQQSEAETP GRTGEGGGSR   250
LLALETLIQN QQLLNLWGCK GRLICYTSVK W                       281
```

linker → (at residue 246); IDR (HAM112) → (at residue 250)

Figure 18

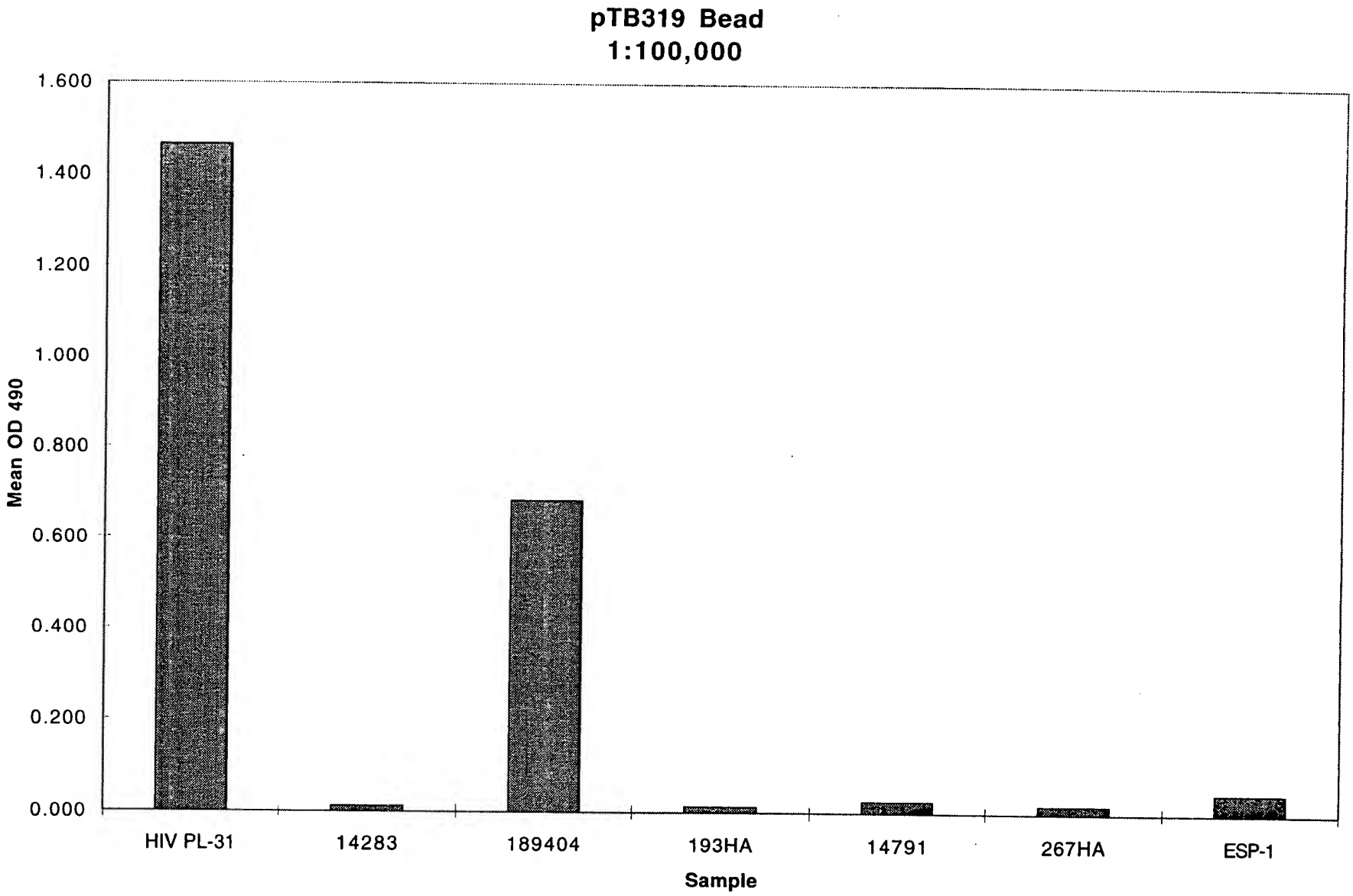

Figure 19

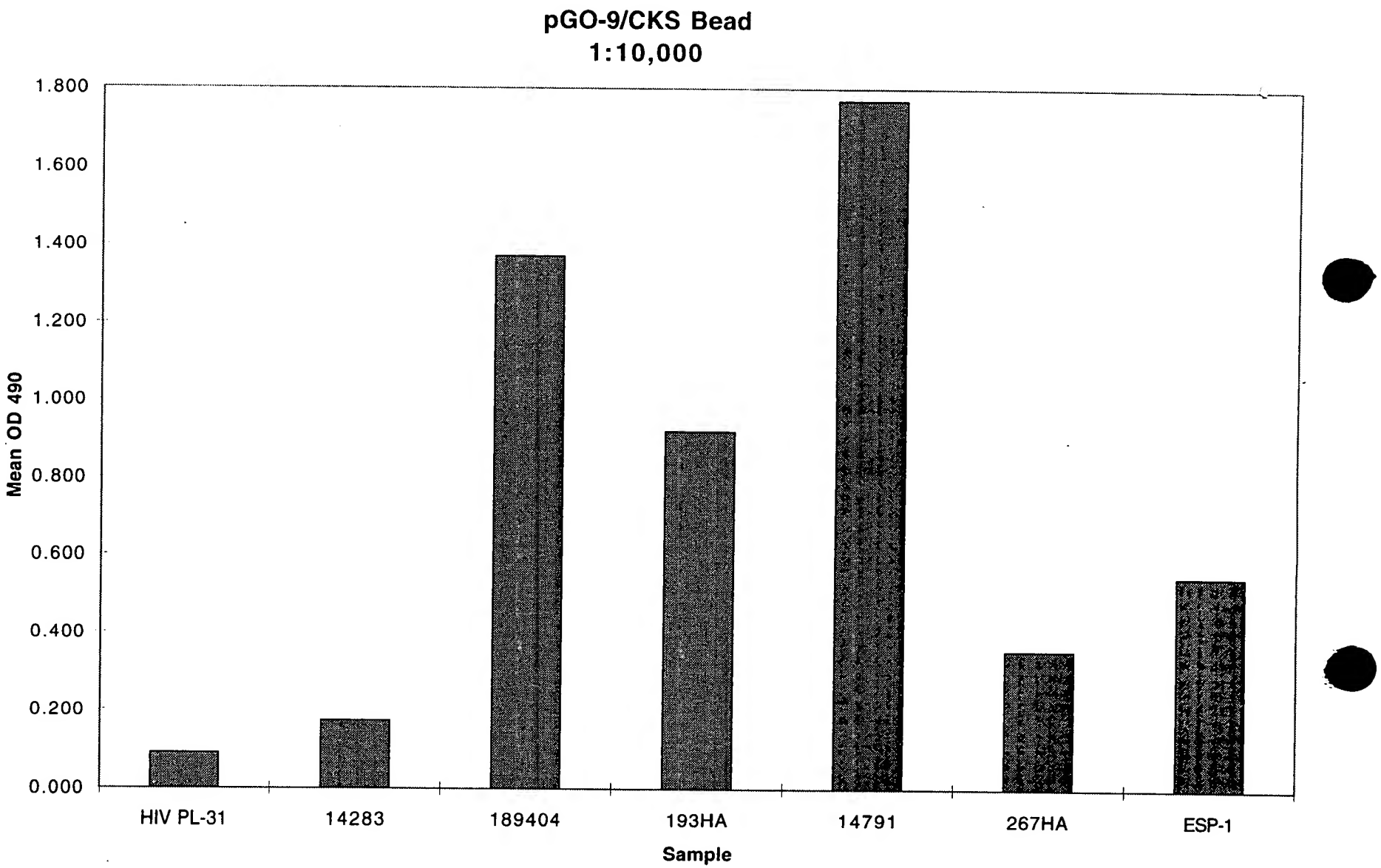

Figure 20

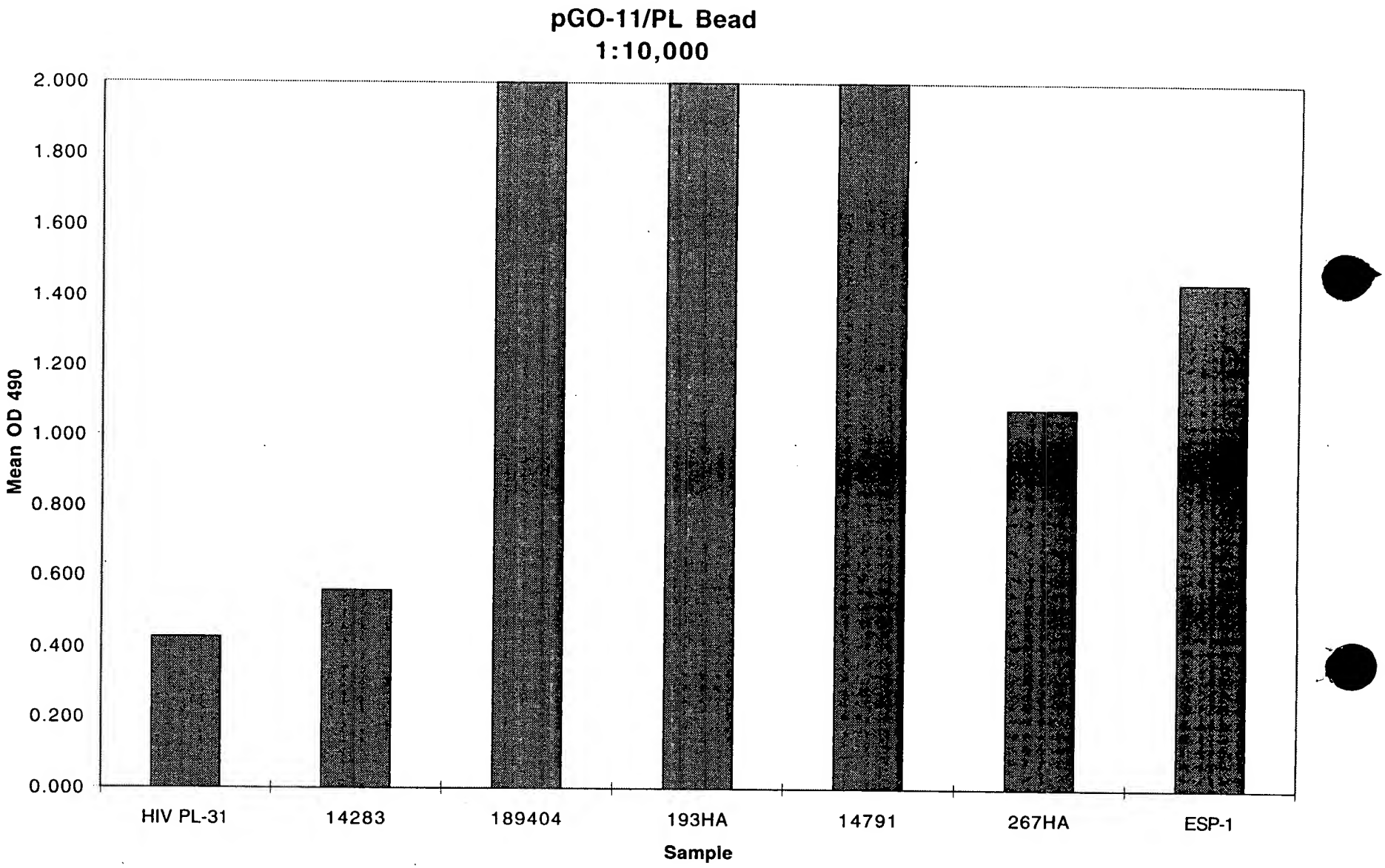
pGO-11/PL Bead
1:10,000
Mean OD 490
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
HIV PL-31
14283
189404
193HA
14791
267HA
ESP-1
Sample

Figure 21

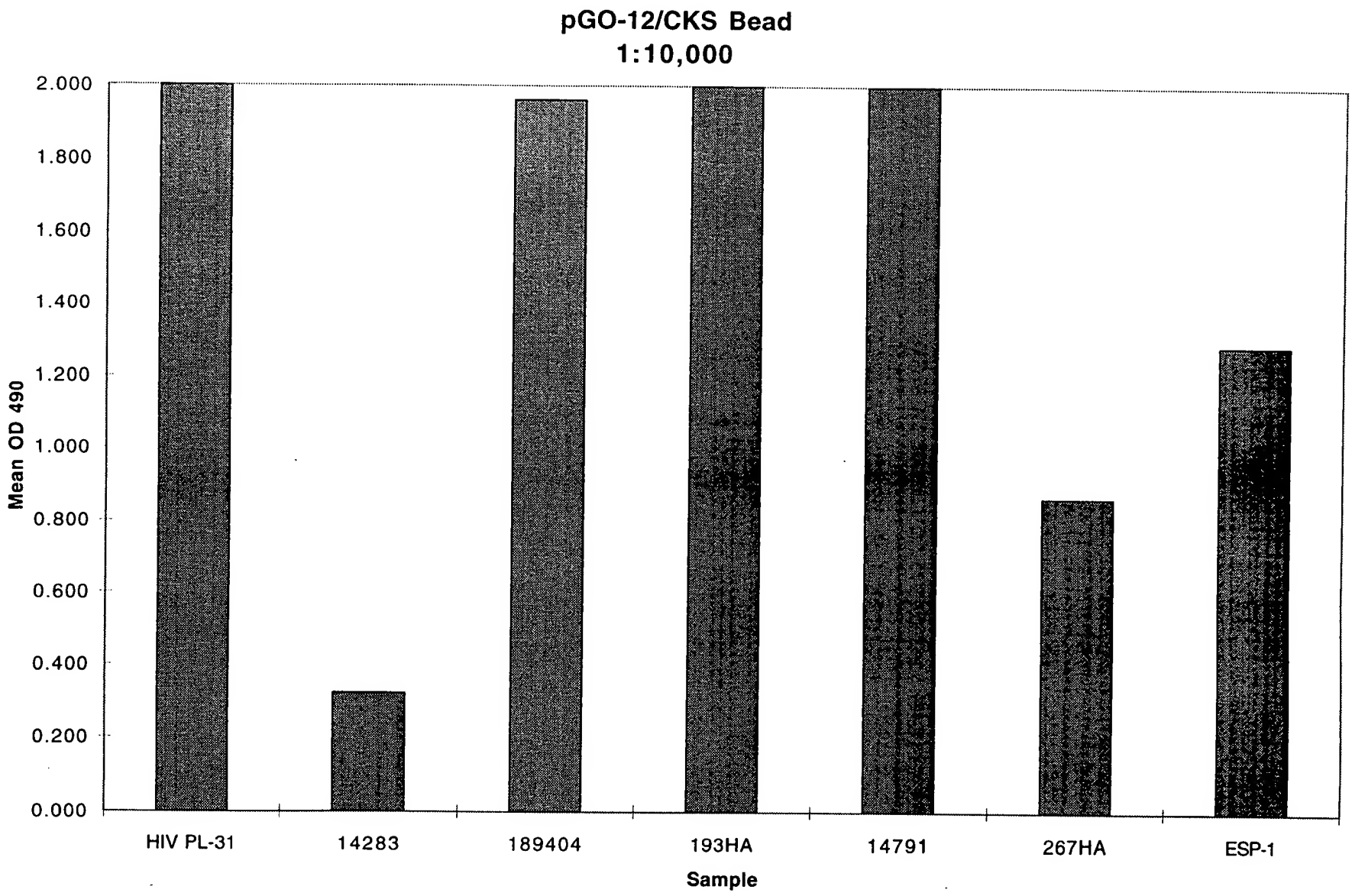

Figure 22

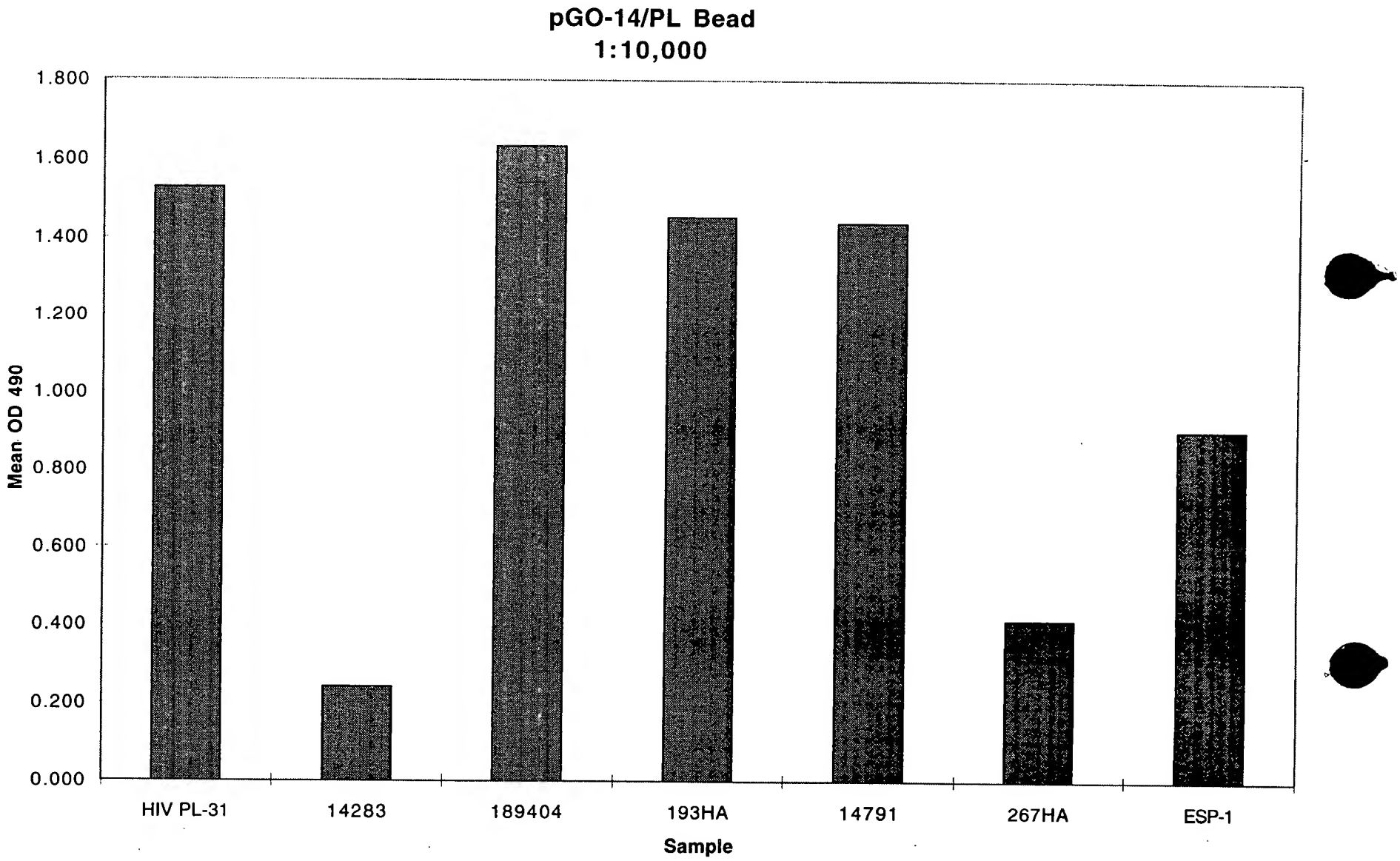

Figure 23